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IMMUNOLOGICAL STUDIES ON CRYPTOCOCCOSIS

REPORT II. STUDIES ON THE SPECIFICITY OF THE POLYSACCHARIDE ANTIGEN OF CRYPTOCOCCUS NEOFORMANS IN EXPERIMENTAL CRYPTOCOCCOSIS

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In the previous report, the author described the extracting and purifying method of polysaccharide fraction from *Cryptococcus neoformans* and its chemical nature. In that report the author also suggested that the immunological diagnosis of cryptococcosis can be applied clinically by performing the precipitin reaction and skin test. Also, the author investigated the degree of manifestation of cross immunological reaction in which the polysaccharide fraction of *Cr. neoformans* was used as an antigen to the animal infected with *Candida* or *Saccharomyces*, both of which presumably possessed a somewhat common antigen as *Cr. neoformans*. The investigation was further carried out as to how the anamnestic reaction would re-appear when a rabbit previously immunized by *Cr. neoformans* was re-immunized after its antibody in blood had disappeared. The latter investigation was also aimed at clarifying the immunization mechanism of *Cr. neoformans*.

METHODS AND MATERIAL

I. Anamnestic immunological reaction:

Animals used: rabbits no. 8, 9, and 10 used in the previous experiment.

Strain used: *Cr. neoformans* no. 2617 and no. 2575 strains.

Method: The rabbits whose antibody titers (precipitin titers) and skin reactions became negative following the previous experiment were re-immunized. *Cr. neoformans* organisms were collected after being cultivated on 3 percent dextrose, Sabrand's agar-agar slant media at 37°C for 48 hours and were heated at 60°C for thirty minutes after being diluted with

physiological saline to contain 1 mg./ml. Each ml. of the obtained solution was injected into the rabbit's ear vein repeatedly. The degree of an elevation of the antibody (precipitin titer) titer in the blood sampled from the rabbits and the degree of manifestation of skin reactions in relationship to the blood antibody-level were frequently examined during the process of the experiment.

RESULTS:

As shown in Illustration 1, the degree and promptness of the manifestation of the precipitin reaction varied depending on the individual differences in the rabbit. But, in each rabbit, some degree of the anamnestic reaction was noticed in their sera, although no anamnestic skin reaction was recognized in any of the rabbits.

II. Cross immunological reaction among the similar organisms:

Animals used: Rabbits weighing approximately two kgs.

Strain used: *Cr. neoformans*, no. 2617 strain, *Candida albicans*, no. 2 strain, *Candida krusei*, no. C-4-1 strain and *Saccharomyces cerevisiae*, no. 2235 strain.

Each organism was collected after being cultivated on three percent dextrose Sabrand's agar-agar slant media at 37°C for 48 hours, and was heated at 60°C for thirty minutes after being diluted with physiological saline to contain 1 mg./ml. One ml. of the obtained solution was injected into each rabbit's ear vein repeatedly.

During the process of the experiment, the antibody-titers of the vaccinated rabbits were frequently measured. Among them, the antibody titer of *C. albicans* immunized rabbit was measured by precipitin reaction in which the polysaccharide fraction extracted from *C. albicans* no. 2 strain was utilized as its antigen (2). On the other hand, the antibody of the *C. krusei* and of the *S. cerevisiae* were titrated by the agglutination reaction. Furthermore, each immunized rabbit's serum was checked for a precipitin reaction by using the polysaccharide fraction extracted from *Cr. neoformans* no. 2617 strain as an antigen.

RESULTS:

Variations of the precipitin titers of *Cr. neoformans* and of the similar organism-immunized rabbits are shown in Illustration 2.

For example, the precipitin reaction in which the polysaccharide fraction extracted from *Cr. neoformans* was used as an antigen was

particularly increased in the serum of *Cr. neoformans* immunized rabbit. On the other hand, in the immunized sera of *C. albicans*, *C. krusei* and of *S. cerevisiae*, the precipitin reaction with *Cr. neoformans* extracted polysaccharide fraction was positive only in the later period of immunization with as high a concentration of the polysaccharide fraction as 1000 mcg./ml.

With regard to the skin reaction, the rabbits immunized by *C. albicans*, *C. krusei* and by *S. cerevisiae* manifested a redness of about (1-5mm) x (1-5mm) in size at 10 mcg./ml. of its concentration.

Variations of the precipitin reactions of *C. albicans* immunized rabbits in which the *C. albicans* extracted polysaccharide fraction and the extracted *Cr. neoformans* were used, are shown in Illustration 3. Also shown in Illustration 3, the precipitin reaction of the serum of the *C. albicans* immunized rabbit against which the polysaccharide fraction extracted from *C. albicans* was matched, was particularly increased. However, its precipitin reaction against which *Cr. neoformans* extracted polysaccharide fraction was matched, was slightly increased only in the later period of the immunization.

DISCUSSION:

Some degree of anamnestic reaction of the cryptococcus infection was provoked by the precipitin reaction. However, it was not marked in its degree and elevation of its antibody titer was extremely slow at the onset when initial immunization was performed. Also, when it was re-immunized following the disappearance of its antibody from the blood, it was as slow and unmarked as the initial one. Generally, its reaction was much slower than that of the bacterial immunization. Moreover, the rate of disappearance of antibody from the blood when re-immunized was much faster than that of the initial immunization. Positive skin reactions were not revealed in any of the three re-immunized rabbits. These findings revealed that re-immunization was quite contrary to that of initial immunization in which the degree of variation in its skin reaction was parallel to those of the precipitin reaction.

The reason for the dulness of the skin reaction found in the serum may be due to the fact that dead organism was used at its re-immunization. Further investigation is required to prove whether or not positive skin reaction can be obtained with the use of the live vaccine. Although it cannot be said without reserve, that such findings as seen in *Cr. neoformans* immunization can be applied to other mycoses, the attitude of the mycoses in general towards immunization should be different from that of bacteria. It can be concluded that some common antigen does exist among *C. albicans*, *C. krusei*, *S. cerevisiae* and *Cr. neoformans*.

since a cross reaction was found among their sera in which the precipitin reaction was used as its antigen. Evans and Sorensen (3) reported on animal experiments in which *Cr. neoformans* and some of the other mycoses proved to possess a common antigen. They used the agglutination and precipitin reaction to prove it.

Salvin (4, 5) also reported the cross reaction between *Cr. neoformans* and other mycoses in which the complement fixation was utilized.

Although it is well recognized that a common antigen exists between *Cr. neoformans* and other similar mycoses, the highly specific reaction against polysaccharide fraction extracted from *Cr. neoformans* was noticed only in the *Cr. neoformans* immunized serum which showed a marked qualitative difference between their reactions.

These findings suggest that the polysaccharide fraction extracted from *Cr. neoformans* can be utilized for the purpose of differential diagnosis.

CONCLUSION:

1. In order to investigate the specificity of the polysaccharide fraction extracted from *Cr. neoformans* no. 2617, cross immunological reactions among the rabbits sera immunized by *Candida albicans*, *Candida krusei*, *Saccharomyces cerevisiae* and *Cr. neoformans* were investigated. The precipitin reaction was utilized for this purpose. As a result of this experiment, a positive cross immunological reaction among them was recognized to a slight degree.

2. It was proved that the anamnestic immunological reaction could be recalled by re-immunizing the rabbit when its antibody titer in blood was decreased and skin reaction became negative sometime after immunization with *Cr. neoformans*.

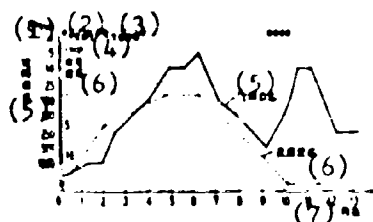


Illustration 1. Variation of the precipitin reaction and skin reaction of the *Cr. neoformans* no. 2575 infected rabbit to which the polysaccharide fraction was utilized as its antigen and of its anamnestic reaction.

Key: 1 — (mcg/ml; 2 — Dead vacc.; 3 — Live vacc.; 4 — mcg; 5 — Precipitin reaction; 6 — Skin reaction; 7 — No. of months.

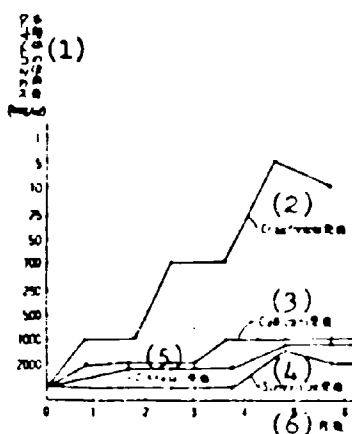


Illustration 2. Variation of the precipitin reaction of the *Cr. neoformans* infected rabbit and of similar mycoses infected rabbits to which the *Cr. neoformans* extracted polysaccharide was utilized as its antigen.

Key: 1 — Amt. of *Cr. neof.* extract. polysacch. fraction (mcg/ml); 2 — *Cr. neof.* immuniz.; 3 — *C. albicans* immuniz.; 4 — *S. Cerevisiae* immuniz.; 5 — *C. krusei* immuniz.; 6 — No. of months.

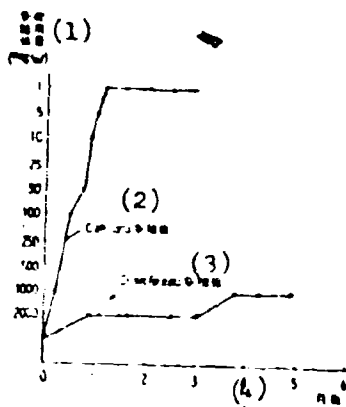


Illustration 3. Variation of the precipitin reaction of the *C. albicans* immunized rabbit to which the *C. albicans* extracted polysaccharide fraction was used as its antigen and of the *Cr. neoformans* immunized rabbit to which extracted *Cr. neoformans* was used as its antigen.

Key: Dose of polysacch. fraction (mcg/ml); 2 -- *C. albicans* P.S.F.; 3 -- *Cr. neoformans* P.S.F.; 4 -- No. of months.

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